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Scanning electron microscopy studies of antennal sensilla of *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae)

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Abstract: In this paper, the external morphology of the antennal sensilla of female and male *Lysiphlebus fabarum* (Marshall) is described using scanning electron microscopy. Seven morphological sensillar types were recognized in both sexes. They are sensilla trichodea (ST), sensilla chaetica type Ⅰ (SCh Ⅰ), sensilla chaetica type Ⅱ (SCh Ⅱ), sensilla basiconica (SB), sensilla coeloconica type Ⅰ (SC Ⅰ), sensilla coeloconica type Ⅱ (SC Ⅱ) and elongated sensilla placodea (SP). The number of sensilla trichodea (ST) is the largest in all sensilla. Sensilla coeloconica type Ⅱ (SC Ⅱ) is only found in female and its number is the least. The shape and structure of most antennal sensilla between male and female adults were not basically different.

Key words: *Lysiphlebus fabarum*; antenna; sensilla; ultrastructure; morphology; scanning electron microscopy

1 INTRODUCTION

The soybean aphid, *Aphis glycines* Matsumura, is widely distributed in the soybean growing regions of China, causing severe damage in Jilin, Liaoning, Heilongjiang, and Inner Mongolia (Wang *et al.*, 1962). If infested by soybean aphid, the soybean yield will be reduced 20% - 30%, even more than 50% (Wang *et al.*, 1996; Wang *et al.*, 1998; Liu and Li, 2005; Shao and Liu, 2009). In recent years, the soybean aphid has been diffused in Australia (Murray and Petter, 2002) and America (Ragsdalea *et al.*, 2004). Our deep investigation of potential biocontrol agents in North-east China shows that *Lysiphlebus fabarum* (Marshall) is an important parasitoid of soybean and plays an important role in natural balance of the soybean aphid population.

The antennae of adult insects have various types of sensilla with different functions and play an important role in various behaviors during adult life. Antennae of female parasitoid (Hymenoptera) are involved in habitat searching, host location, host examination, host recognition, host acceptance, oviposition, host discrimination and mating behavior (Weseloh, 1972; Dahms, 1984; Bin and Vinson, 1986; Vinson *et al.*, 1986; Bin *et al.*, 1989; Isidoro *et al.*, 1996). Antennae of male parasitoid (Hymenoptera) are involved in courtship behavior (Isidoro and Bin, 1995). In laboratory, we observed that the female of *L. fabarum* always touches and beats the soybean aphid with its terminal antennomeres; then it will choose the 2 or 3 instar soybean aphid to parasitize. The antennae just like a medium between the parastoid and soybean aphid, thus studying antennal morphology, sensilla and structures will, therefore, help to understand the behavior variations of the individual species (Olson and Andow, 1993; Ochieng *et al.*, 2000). To provide a solid basis for research on parasitic behavior of *L. fabarum*, we described the morphology of both female and male antennal sensilla using scanning electron microscopy. The location, abundance, and distribution of the sensilla are also observed.

2 MATERIALS AND METHODS

2.1 Insects

Females and males of *Lysiphlebus fabarum* (Marshall) used in this study were reared in our laboratory (temperature: 25℃; relative humidity: 60% ± 10%; photophase: L∶D = 16∶8) from mummies of *Aphis glycines* (Matsumura) collected in Liaoning Province, China.

2.2 Electron microscopy

Antennae of *L. fabarum* were carefully excised from the antennal sockets with fine forceps at 10 × under a stereomicroscope (Laica 12.5). The antennae were first kept in 70% ethanol for 24 h and then dehydrated in a graded alcohol series of 75%,

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80%, 85%, 90% and 99.9% (van Baren *et al.*, 1996) in each case for 1 h. The antennae were later mounted on a holder using double-sided adhesive tape and sputter-coated with gold. Examinations were done with a FEI Quanta 200F SEM at 12.5 kV. Sensilla on the dorsal and ventral surfaces of the antennae of *L. fabarum* were identified, counted, and measured. At least 6 antennae were examined from separate female and male wasps.

Sensilla on the dorsal and ventral surfaces of the antennae of *L. fabarum* were identified, counted, and measured. Final measurements (μm) were obtained by using mean measurements from photomicrographs of at least 12 individual sensilla of the same type.

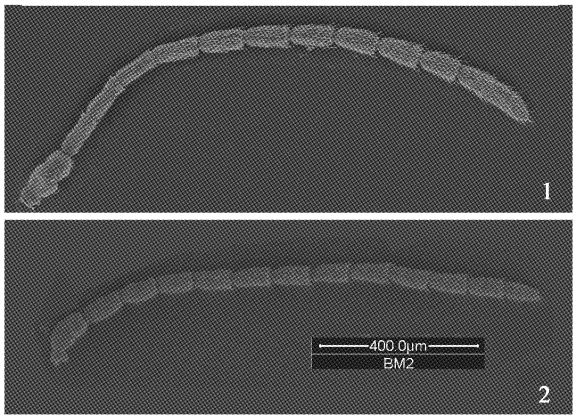
2.3 Terminology

The terminology of insect antennae is somewhat inconsistent, and sensillar types may have different names though similar in shape and distribution. Even among the different species in one family, sensillar types possess the similar morphology, but have the varying names. Our inference from the published photomicrographs and using the morphological characters such an presence and position of pores as bases for identification, among them are Chapman (1982, 1998), Wibel *et al.* (1984), Isidoro *et al.* (1996), Amornsak *et al.* (1998), Pettersson *et al.* (2001), Ryan (2002) and Bleeker *et al.* (2004).

3 RESULTS

3.1 General description of antennae

The antennae of both male and female *L. fabarum* are threadlike in shape. In general, the flagellum of female is composed of 11 sub-segments (Fig. 1), but sometimes there are 12 sub-segments; the flagellum of male is composed of 12 sub-segments (Fig. 2). The total length of male and female is 1 333.3 μm and 1 431.8 μm, and the diameter is 44.2 μm and 46.6 μm, respectively.



Figs. 1 – 2 The antenna of *Lysiphlebus fabarum*
1: Female (same scale as Fig. 2); 2: Male.

3.2 Sensillar types

Seven sensillar types were found on the antennae of both males and females of *L. fabarum*. We identified them as sensilla trichodea (ST), sensilla chaetica (SCh), sensilla basiconica type Ⅰ (SB Ⅰ), sensilla basiconica type Ⅱ (SB Ⅱ), sensilla coeloconica type Ⅰ (SC Ⅰ), sensilla coeloconica type Ⅱ (SC Ⅱ) and elongated sensilla placodea (PS). The distribution of the different sensillar types on each antennal segment is shown in Table 1.

Table 1 Abundance and distribution of different sensilla on the antennae of female and male *Lysiphlebus fabarum* (Marshall)

Sensilla	Sex	Antennal segment															Total
		Rd	Sc	Pd	Fn1	Fn2	Fn3	Fn4	Fn5	Fn6	Fn7	Fn8	Fn9	Fn10	Fn11	En12	
ST	Female	–	18 ±1	40 ±2	54 ±2	52 ±2	46 ±2	53 ±2	59 ±2	71 ±2	74 ±2	78 ±3	74 ±2	79 ±3	98 ±3	142 ±4	938 ±4
	Male	–	9 ±1	18 ±1	33 ±2	39 ±2	43 ±2	48 ±2	55 ±2	60 ±2	78 ±2	74 ±2	73 ±2	81 ±3	86 ±3	96 ±3	793 ±4
PS	Female	–	–	–	3 ±1	4 ±1	6 ±1	5 ±1	7 ±1	8 ±1	7 ±1	9 ±1	9 ±1	8 ±1	7 ±1	15 ±2	88 ±3
	Male	–	–	–	5 ±1	7 ±1	9 ±1	8 ±1	9 ±1	8 ±1	10 ±1	9 ±1	13 ±1	10 ±1	9 ±1	7 ±1	104 ±2
SCh Ⅰ	Female	–	–	–	–	1 ±0	3 ±0	4 ±0	6 ±1	6 ±1	8 ±1	9 ±1	9 ±1	10 ±1	14 ±1	15 ±1	85 ±2
	Male	–	–	–	–	1 ±0	3 ±0	7 ±1	9 ±1	11 ±1	10 ±1	11 ±1	12 ±1	13 ±1	12 ±1	11 ±1	96 ±2
SCh Ⅱ	Female	31 ±2	9 ±1	3 ±0	–	–	–	–	–	–	–	–	–	–	–	–	43 ±2
	Male	19 ±1	4 ±0	6 ±1	–	–	–	–	–	–	–	–	–	–	–	–	29 ±2
SB	Female	–	–	–	–	2 ±0	2 ±0	4 ±1	4 ±1	5 ±1	5 ±1	7 ±1	7 ±1	8 ±1	7 ±1	13 ±1	64 ±2
	Male	–	–	–	1 ±0	1 ±0	2 ±0	5 ±1	4 ±1	4 ±1	5 ±1	6 ±1	6 ±1	5 ±1	6 ±1	10 ±1	55 ±2
SC Ⅰ	Female	–	–	–	–	–	–	–	–	–	–	1 ±0	2 ±0	3 ±0	3 ±0	4 ±1	13 ±1
	Male	–	–	–	–	–	–	–	–	–	–	1 ±0	1 ±0	2 ±0	2 ±0	3 ±0	9 ±1
SC Ⅱ	Female	–	–	–	–	–	–	–	–	–	–	1 ±0	2 ±0	1 ±0	1 ±0	1 ±0	6 ±1
	Male	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Data are presented as mean ± *SE* (n = 6).

3.3 Morphology and structure of sensilla

3.3.1 Sensilla trichodea (ST): Sensilla trichodea (ST) are present on all antennal segments of both males and females (Figs. 3, 7, 10, 11). These sharply pointed structures are inclined and slightly curved toward the apex of the segment (Fig. 14). The base is inserted in a flexible socket, which is slightly elevated above the cuticle. Surface of each sensillum is smooth but exhibits longitudinal grooves laterally and small pits in basal part (Figs. 4, 15). The sensilla vary in length from approximately (15.3 ± 0.5) μm to (24.1 ± 0.3) μm in females, and (10.5 ± 0.5) μm to (19.8 ± 0.5) μm in males.

3.3.2 Sensilla chaetica type Ⅰ (SCh Ⅰ): These sensilla are present in both sexes and are distributed in the mid- and distal-portions of each flagellum sub-segment (Figs. 3, 10, 11). They are characterized by a smooth surface and project slightly more perpendicularly with respect to the axis of the antenna than sensilla trichodea. The tips of sensilla are blunt and without pores. The length of these sensilla is about (11.6 ± 0.5) μm in both males and females.

3.3.3 Sensilla chaetica type Ⅱ (SCh Ⅱ): These sharply pointed sensilla are found only on the scape and radicular in both sexes (Figs. 7, 16) and fewer in number compared with other sensillar types. This type of sensillum is similar in shape to sensilla trichodea but varies in length, from (4.8 ± 0.3) μm to (18.6 ± 0.4) μm in females and from (3.4 ± 0.4) μm to (17.8 ± 0.3) μm in males. They have a smooth surface.

3.3.4 Sensilla basiconica (SB): Sensilla basiconica (SB) are found on all flagellar sub-segments of both sexes and present more often in the distal-portions, especially evident at the distal portion of the last sub-segment (Figs. 3, 9, 10, 11). They are characterized by a smooth surface (Fig. 5) and have a few grooves on the surface (Fig. 12). These sensilla are project more perpendicularly with respect to the axis of the antenna than sensilla trichodea and sensilla coeloconica type Ⅰ. Because of their length and orientation, the tips of these sensilla are well above the level of other sensilla. The tips of these sensilla have a pore (Figs. 5, 6) in females, and have no pore (Fig. 13) in males. The length of this sensillum is about (24.6 ± 0.5) μm in both sexes.

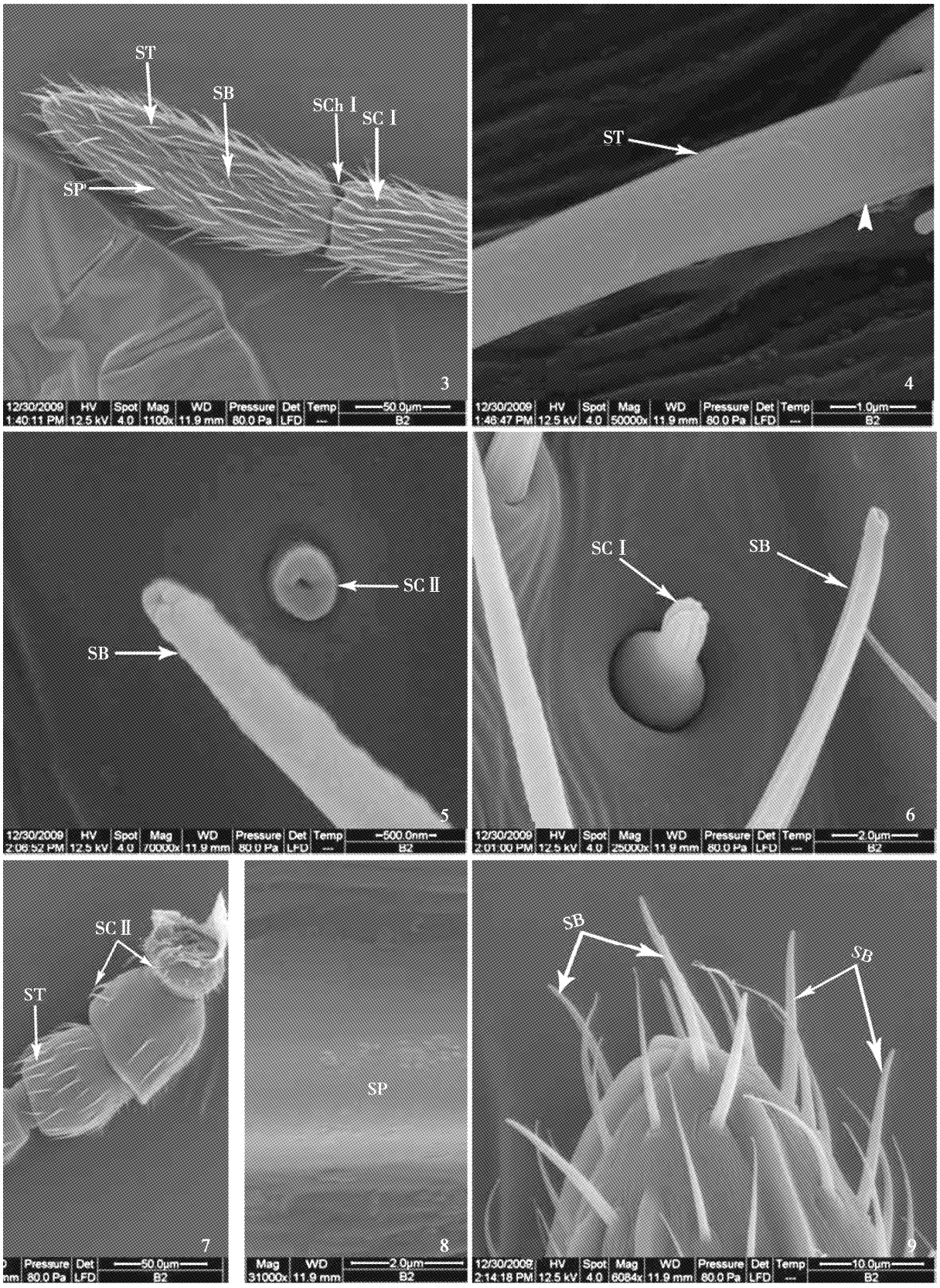
3.3.5 Sensilla coeloconica type Ⅰ (SC Ⅰ): Sensilla coeloconica type Ⅰ are found on the dorsal surface of the flagellar sub-segment of both sexes (Figs. 3, 11). They are located in sunken depressions and surrounded by a doughnut-shaped ring of (2.5 ± 0.1) μm in diameter of the females and (2.6 ± 0.1) μm in males. The length of this sensillum is (1.6 ± 0.4) μm and (2.8 ± 0.1) μm in females and males, respectively. The diameter of the peg (the tip of this sensillum) in females is (1.1 ± 0.1) μm and (0.8 ± 0.1) μm in males, respectively. The peg consists of 9 finger-like projections join together (Figs. 6, 14).

3.3.6 Sensilla coeloconica type Ⅱ (SC Ⅱ): These sensilla are only present in females. This sensillum consists of a button-like projection, and the diameter measures (0.7 ± 0.0) μm. The tip of this sensillum has a pore in the center (Fig. 5), and the diameter of the pore is (0.2 ± 0.0) μm. The sensillum is surrounded by a donut-shaped ring of (1.0 ± 0.0) μm in diameter.

3.3.7 Sensilla placodea (SP): The placoid sensilla are numerous on all flagellar sub-segments of both sexes (Figs. 3, 11). In the males, the length and width of the placoid sensilla is (85.1 ± 1.4) μm and (2.7 ± 0.2) μm, respectively. In the females, the length and width of the placoid sensilla is (87.6 ± 1.2) μm and (2.4 ± 0.2) μm, respectively. These sensilla are slightly elevated above the antennal surface and are located between the rows of sensilla trichodea and sensilla basiconica (Fig. 10). They are distributed equally around the antennomere and aligned generally parallel to the antennal longitudinal axis. This type of sensillum has a smooth and porous surface (Fig. 8).

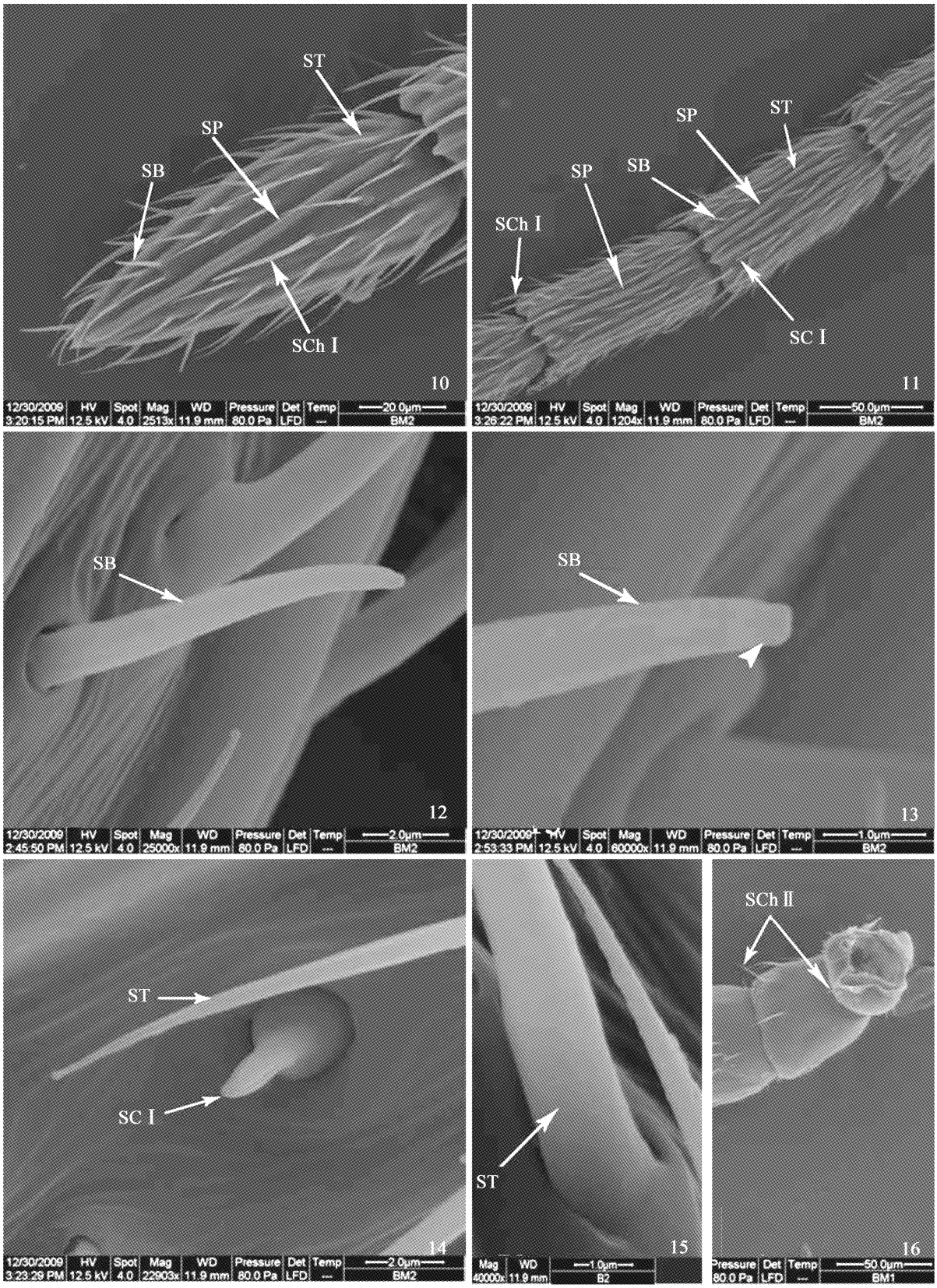
4 DISCUSSION

Based on SEM, we found that *L. fabarum* has seven types of antennal sensilla. The external morphology, types and distribution of sensilla on the antennae of male and female *L. fabarum* recorded in this study are generally in conformity with those reported for other parasitoid species (Wibel *et al.*, 1984; Olson and Andow, 1993; Isidoro *et al.*, 1996; Pettersson *et al.*, 2001; Bleeker *et al.*, 2004), including those species that belong to the same family (Braconidae) or superfamily (Ichneumonoidea) (Norton and Vinson, 1974; Navasero and Elzen, 1991; Barbarossa *et al.*, 1998; Ochieng *et al.*, 2000; Chen *et al.*, 2004; Li and Bai, 2004; Bourdais *et al.*, 2006; Dong and Zhang, 2006). A detailed analysis of SEM microphotographs of parasitoid wasps antennal sensilla indicates that similarity of sensilla between species is highly dependent on how closely related they are (Bleeker *et al.*, 2004). We also found the antennal sensilla of *L. fabarum* show more similarity to those of *Aphidius rhopalosiphi* (Bourdais *et al.*, 2006), which also belongs to Aphidiinae.



Figs. 3 – 9　Sensilla of antennae of *Lysiphlebus fabarum*, female

3: Sensilla on terminal two flagella. Sensilla trichodea (ST); Sensilla chaetica type Ⅰ (SCh Ⅰ); Sensilla basiconica (SB); Sensilla coeloconica type Ⅰ (SC Ⅰ); Sensilla placodea (SP). 4: Sensilla trichodea (ST) showing the smooth surface with small pits and the grooves on the lateral (arrowhead). 5: Sensilla basiconica (SB) showing the pore and sensilla coeloconica type Ⅱ (SC Ⅱ) showing the terminal pore. 6: Sensilla basiconica (SB) and sensilla coeloconica type Ⅰ(SCⅠ) showing finger-like projections join together. 7: Sensilla trichodea (ST) on the pedicel, sensilla chaetica type Ⅱ (SChⅡ) on the radicula and scape. 8: Sensilla placodea (SP) showing the smooth surface. 9: Sensilla basiconica (SB) on the distal portion of the terminal flagellum.



Figs. 10 – 16 Sensilla of antennae of *Lysiphlebus fabarum*, male

10: Sensilla on the terminal flagellum segment. Sensilla trichodea (ST); Sensilla chaetica type Ⅰ (SCh Ⅰ); Sensilla basiconica (SB); Sensilla placodea (SP). 11: The 10th and 11th flagellum segments showing sensilla trichodea (ST); Sensilla Chaetica type Ⅰ (SCh Ⅰ); Sensilla basiconica (SB); Sensilla coeloconica type Ⅰ (SC Ⅰ); Sensilla placodea (SP). 12: Sensilla basiconica (SB). 13: Sensilla basiconica (SB) showing the tip without pore. 14: Sensilla trichodea (ST) and sensilla coeloconica type Ⅰ (SC Ⅰ) with finger-like projections join together. 15: Sensilla trichodea (ST) showing smooth surface. 16: Sensilla chaetica type Ⅱ (SCh Ⅱ) on the radicula and scape.

In *L. fabarum*, the length of female antennae is slightly longer than that of male. The sensilla coeloconia (SC Ⅱ) is only found in females, and it is absent in males. The shape of apex of sensilla basiconica is different between the sexes. The tips of sensilla basiconica have a pore (Figs. 5, 6) in females, but no pore is found (Fig. 13) in males. Other from differences above, there is no significant sexual differences in the distribution and abundance of the rest sensillar types on the antennae of *L. fabarum*. Sexual differences in types and location of sensilla are broadly present in other parasitoids that belong to the Chalcidoidea and Platygasteroidea, and these differences are thought to be associated with sex-specific differences in behavior, *e. g.*, courtship and host recognition (Cave and Gaylor, 1987; Amornsak *et al.*, 1998; van Baaren *et al.*, 1999). However, in some parasitic Hymenoptera, such as, *Cotesia rubecula* (Bleeker *et al.*, 2004) and *Microplitis croceipes* (Ochieng *et al.*, 2000), sexual differences in types and location of sensilla were absent.

Based on structures or shapes of these antennal sensilla, some are deduced to be mechanoreceptors (Keil, 1999) and thermo- or hygroreceptor (Altner *et al.*, 1983). Some have gustatory function (Barbarossa *et al.*, 1998) and olfactory (Steinbrecht, 1997; Keil, 1999). However, more study need to be conducted to confirm these deductions.

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豆柄瘤蚜茧蜂触角感受器的扫描电镜观察

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摘要: 利用扫描电镜对豆柄瘤蚜茧蜂 *Lysiphlebus fabarum* 的触角感受器进行了观察, 发现有 7 种感受器, 分别为毛形感受器、刺形感受器Ⅰ型、刺形感受器Ⅱ型、锥形感受器、腔锥形感受器Ⅰ型、腔锥形感受器Ⅱ型和板形感受器。毛形感受器是数量最多的感器; 除了腔锥形感受器Ⅱ型只在雌虫触角发现外, 雌、雄触角感受器的类型和结构没有较大的差别, 并且腔锥形感受器Ⅱ是数量最少的感器。

关键词: 豆柄瘤蚜茧蜂; 触角; 感受器; 超微结构; 形态; 扫描电镜

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